

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C07K 14/435, A61K 39/00		A1	(11) International Publication Number: <b>WO 99/58564</b> (43) International Publication Date: 18 November 1999 (18.11.99)
(21) International Application Number: PCT/NO99/00141 (22) International Filing Date: 30 April 1999 (30.04.99) (30) Priority Data: 19982098 8 May 1998 (08.05.98) NO (71) Applicant (for all designated States except US): NORSK HYDRO ASA [NO/NO]; N-0240 Oslo (NO). (72) Inventors; and (75) Inventors/Applicants (for US only): GAUDERNACK, Gustav [NO/NO]; Anthon Walles vei 17 A, N-1300 Sandvika (NO). ERIKSEN, Jon, Amund [NO/NO]; Bjørntvedt gt. 37, N-3916 Porsgrunn (NO). MØLLER, Mona [NO/NO]; Skrukkerødtoppen 8, N-3925 Porsgrunn (NO). (74) Agent: LILLEGRAVEN, Rita; Norsk Hydro ASA, N-0240 Oslo (NO).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND UBIQUITIN-B AND THEIR USE (57) Abstract  Frameshift Mutants $\beta$ -Amyloid precursor peptides and mutant ubiquitin-B associated with Alzheimer's disease and Down syndrome eliciting T cellular immunity for use in compositions for the treatment and/or prophylaxis of Alzheimer's disease and/or Down syndrome.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TC	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND UBIQUITIN-B AND THEIR USE

5 The present invention relates to peptides for treatment  
and/or prophylaxis of Alzheimer's disease and Down  
syndrome.

Alzheimer's disease and treatment of Down syndrome are both  
10 associated with frameshift mutations occurring at the  
transcriptional level or by posttranscriptional editing of  
RNA during the encoding of  $\beta$ -Amyloid precursor protein  
( $\beta$ APP) and ubiquitin-B (Ubi-B). Such frameshift mutations  
give rise to mutant  $\beta$ APP and Ubi-B protein products which  
15 are characterised by aberrant protein sequences at the  
carboxyl terminus. Peptides covering, either completely or  
parts of, the aberrant parts of mutant  $\beta$ APP or Ubi-B  
protein products elicit T cellular immunity and can  
therefore be useful in compositions for the treatment of  
20 Alzheimer's disease and Down syndrome. Further the peptides  
of this invention can be used as a prophylactic  
anti-Alzheimer's disease vaccine.

The invention also relates to DNA sequences encoding  
25 peptides corresponding to aberrant  $\beta$ APP and Ubi-B protein  
sequences found in Alzheimer's disease and Down syndrome  
patients, and to vectors comprising at least one insertion  
site containing a DNA sequence encoding at least one such  
peptide.

30 Further the invention relates to methods for the treatment  
and/or prophylaxis of Alzheimer's disease by administration  
of at least one mutant  $\beta$ APP and/or Ubi-B peptide or a  
recombinant virus vector comprising at least one insertion

site containing a DNA sequence encoding at least one mutant  $\beta$ APP and/or one mutant Ubi-B peptide.

The present invention represents a development of a  
5 treatment and/or prophylaxis for Alzheimer's disease based  
on the use of peptides to generate activation of the T  
cellular arm of the body's own immune system against cells  
producing mutant  $\beta$ APP and Ubi-B protein products associated  
with Alzheimer's disease.

10 The development and use of the methods for treatment of  
Alzheimer's disease may also be directly applicable for  
treatment of patients with Down syndrome.

15 Technical Background

Peptides corresponding to aberrant protein sequences  
resulting from frameshift mutations in genes in cancer  
20 cells elicit specific T cellular immunity and can be used  
as anti-cancer vaccines (ref. Norwegian patent applications  
filed at the same date as the present application by Norsk  
Hydro ASA). In the same manner peptides corresponding to  
aberrant protein sequences resulting from frameshift  
25 mutations associated with other diseases can be used to  
develop treatments of that diseases based on generation of  
specific T cellular immunity.

30 Frameshift mutations result in completely new amino acid  
sequences in the C-terminal part of the proteins,  
prematurely terminating where a novel stop codon appears.  
This results in two important consequences:

1) The truncated protein resulting from the frameshift is  
35 generally nonfunctional, in most cases resulting in  
"knocking out" of an important cellular function. Aberrant

proteins may also gain new functions such as the capacity to aggregate and form plaques. In both cases the frameshift results in disease.

5     2) The aberrant new C-terminal amino acid sequence resulting from the frameshift is foreign to the body. It does not exist prior to the mutation, and it only exists in cells having the mutation.

10    Since the mutant part of the proteins are completely novel and therefore foreign to the immune system of the carrier, they may be recognized by T-cells in the repertoire of the carrier. So far, nobody has focused on this aspect of frameshift mutations, and no reports exist  
15    on the characterization of frameshift peptides from coding regions of proteins as antigens. This concept is therefore novel and forms the basis for developing vaccines based on these sequences. It follows that such vaccines may also be used prophylactically in persons who inherit defective  
20    genes or in other ways are disposed for frameshift mutations. Such vaccines will therefore fill an empty space in the therapeutic armament against inherited forms of disease.

25    It has been shown that single amino acid substitutions in intracellular "self"-proteins may give rise to tumour rejection antigens, consisting of peptides differing in their amino acid sequence from the normal peptide. The T cells which recognise these peptides in the context of the  
30    major histocompatibility (MHC) molecules on the surface of the tumour cells, are capable of killing the tumour cells and thus rejecting the tumor from the host.

35    In contrast to antibodies produced by the B cells, which typically recognise a free antigen in its native conformation and further potentially recognise almost any

site exposed on the antigen surface, T cells recognise an antigen only if the antigen is bound and presented by a MHC molecule. Usually this binding will take place only after appropriate antigen processing, which comprises a  
5 proteolytic fragmentation of the protein, so that the resulting peptide fragment fits into the groove of the MHC molecule. Thereby T cells are enabled to also recognise peptides derived from intracellular proteins. T cells can thus recognise aberrant peptides derived from anywhere in  
10 the cells, in the context of MHC molecules on the surface of the cells, and can subsequently be activated to eliminate the cells harbouring the aberrant proteins.

M. Barinaga, Science, 257, 880-881, 1992 offers a short  
15 review of how MHC binds peptides. A more comprehensive explanation of the Technical Background for this Invention may be found in D. Male et al, Advanced Immunology, 1987, J.B. Lippincott Company, Philadelphia. Both references are hereby included in their entirety.

20 The MHC molecules in humans are normally referred to as HLA (human leukocyte antigen) molecules. They are encoded by the HLA region on the human chromosome No 6.

25 The HLA molecules appear as two distinct classes depending on which region of the chromosome they are encoded by and which T cell subpopulations they interact with and thereby activate primarily. The class I molecules are encoded by the HLA A, B and C subloci and they primarily activate CD8+  
30 cytotoxic T cells. The HLA class II molecules are encoded by the DR, DP and DQ subloci and primarily activate CD4+ T cells, both helper cells and cytotoxic cells.

35 Normally every individual has six HLA Class I molecules, usually two from each of the three groups A, B and C. Correspondingly, all individuals have their own selection

of HLA Class II molecules, again two from each of the three groups DP, DQ and DR. Each of the groups A, B, C and DP, DQ and DR are again divided into several subgroups. In some cases the number of different HLA Class I or II molecules is reduced due to the overlap of two HLA subgroups.

All the gene products are highly polymorphic. Different individuals thus express distinct HLA molecules that differ from those of other individuals. This is the basis for the difficulties in finding HLA matched organ donors in transplantations. The significance of the genetic variation of the HLA molecules in immunobiology is reflected by their role as immune-response genes. Through their peptide binding capacity, the presence or absence of certain HLA molecules governs the capacity of an individual to respond to peptide epitopes. As a consequence, HLA molecules determine resistance or susceptibility to disease.

T cells may control the development and growth of cells producing aberrant proteins by a variety of mechanisms. Cytotoxic T cells, both HLA class I restricted CD8+ and HLA Class II restricted CD4+, may directly kill cells carrying the appropriate antigens. CD4+ helper T cells are needed for cytotoxic CD8+ T cell responses as well as for antibody responses, and for inducing macrophage and LAK cell killing.

A requirement for both HLA class I and II binding is that the peptides must contain a binding motif, which usually is different for different HLA groups and subgroups. A binding motif is characterised by the requirement for amino acids of a certain type, for instance the ones carrying large and hydrophobic or positively charged side groups, in definite positions of the peptide so that a narrow fit with the pockets of the HLA binding groove is achieved. The result of this, taken together with the peptide length restriction

of 8-10 amino acids within the binding groove, is that it is quite unlikely that a peptide binding to one type of HLA class I molecules will also bind to another type. Thus, for example, it may very well be that the peptide binding motif  
5 for the HLA-A1 and HLA-A2 subgroups, which both belong to the class I gender, are as different as the motifs for the HLA-A1 and HLA-B1 molecules.

For the same reasons it is not likely that exactly the same  
10 sequence of amino acids will be located in the binding groove of the different class II molecules. In the case of HLA class II molecules the binding sequences of peptides may be longer, and it has been found that they usually contain from 10 to 16 amino acids, some of which, at one or  
15 both terminals, are not a part of the binding motif for the HLA groove.

However, an overlap of the different peptide binding motifs of several HLA class I and class II molecules may occur.  
20 Peptides that have an overlap in the binding sequences for at least two different HLA molecules are said to contain "nested T cell epitopes". The various epitopes contained in a "nested epitope peptide" may be formed by processing of the peptide by antigen presenting cells and thereafter be  
25 presented to T cells bound to different HLA molecules. The individual variety of HLA molecules in humans makes peptides containing nested epitopes more useful as general vaccines than peptides that are only capable of binding to one type of HLA molecule.

30 Effective vaccination of an individual can only be achieved if at least one type of HLA class I and/or II molecule in the patient can bind a vaccine peptide either in it's full length or as processed and trimmed by the patient's own  
35 antigen presenting cells.



The usefulness of a peptide as a general vaccine for the majority of the population increases with the number of different HLA molecules it can bind to, either in its full length or after processing by antigen presenting cells.

5

In order to use peptides derived from an aberrant protein resulting from mutational events in cells as vaccines or therapeutic agents to generate CD4+ and/or CD8+ T cells, it is necessary to investigate the mutant protein in question and identify peptides that are capable, eventually after processing to shorter peptides by the antigen presenting cells, to stimulate T cells.

10

15 Definition of Problem solved by the Invention.

At present no drug or other treatment is able to eliminate the cells producing the aberrant proteins responsible for the degenerative process in the brain of patients afflicted with Alzheimers disease. There is a continuing need for treatment and prophylaxis of Alzheimer's disease. The present invention will contribute to supply new peptides that can have use as treatment and prophylaxis of Alzheimer's disease. Our approach is aimed at directly eliminating the cells responsible for the degenerative process.

20

25

The peptides may also be applicable for treatment of patients with Down syndrome.

30

Definition of the Invention

A main object of the invention is to obtain peptides corresponding to peptide fragments of aberrant  $\beta$ APP and

35

Ubi-B proteins found in Alzheimer's disease and/or Down syndrome patients which can be used to stimulate T cells.

Another main object of the invention is to develop a  
5 treatment for Alzheimer's disease based on the T cell immunity which may be induced in patients by stimulating their T cells either *in vivo* or *ex vivo* with the peptides according to the invention.

10 A third main object of the invention is to develop a vaccine to prevent the establishment of Alzheimer's disease based solely or partly on peptides corresponding to peptides of the present invention which can be used to generate and activate T cells which produce cytotoxic T  
15 cell immunity against cells producing the mutant  $\beta$ APP and mutant Ubi-B proteins.

A fourth main object of the invention is to design a treatment or prophylaxis for Alzheimer's disease  
20 specifically adapted to a human individual in need of such treatment or prophylaxis, which comprises administering at least one peptide according to this invention.

A fifth object of the invention is to obtain a treatment  
25 for patients with Down syndrome using the same methods as for the treatment of Alzheimer's disease.

These and other objects of the invention are achieved by the attached claims.

30

Frameshift mutations can occur at the gene level, transcriptional level or by posttranscriptional editing of RNA and result in premature stop codons and therefore a deletion of sometimes large parts of the proteins. Aberrant  
35 proteins arising from frameshift mutations have generally

not been considered to be immunogenic and have therefore not been considered as targets for immunotherapy. Thus it has now surprisingly been found that a group of new peptides corresponding to aberrant proteins resulting from frameshift mutations associated with Alzheimer's disease and Down syndrome are useful for eliciting T cell responses against cells producing such aberrant proteins.

Genes containing a mono nucleoside base repeat sequence, for example of deoxyadenosine bases, or a di-nucleoside base repeat sequence, for example of deoxycytosine-deoxythymidine units, are susceptible to frameshift mutations. The frameshift mutations occur, respectively, either by insertion of one or two of the mono-nucleoside base residue or of one or two of the di-nucleoside base unit in the repeat sequence, or by deletion of one or two of the mono-nucleoside base residue or of one or two of the di-nucleoside base unit from the repeat sequence. A frameshift mutation will from the point of mutation encode a protein with a new and totally different amino acid sequence as compared to the normal protein. This mutant protein with the new amino acid sequence at the carboxy end will be specific for all cells in which such frameshift mutations have occurred.

In the remainder of this specification and claims the denomination frameshift mutant peptides will comprise such proteins and peptide fragments thereof.

These peptides are at least 8 amino acids long and correspond to frameshift mutant  $\beta$ APP and/or Ubi-B protein sequences associated with Alzheimer's disease and/or Down syndrome.

A peptide according to this invention is characterised in that it

5 a) is at least 8 amino acids long and is a fragment of a mutant  $\beta$ APP and/or Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease or Down syndrome;

and

10

b) consists of at least one amino acid of the mutant part of the mutant  $\beta$ APP and/or Ubi-B protein;

and

15

c) comprises 0-10 amino acids corresponding to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and may further extend to the carboxyl terminus of the mutant part of the protein as determined by a new stop codon generated by the relevant frameshift mutation;

20

and

25 d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

The peptides of this invention contain preferably 8-25, 30 9-20, 9-16, 8-12 or 20-25 amino acids. They may for instance contain 9, 12, 13, 16 or 21 amino acids.

It is most preferred that the peptides of the present invention are at least 9 amino acids long, for instance

9-18 amino acids long, but due to the processing possibility of the antigen presenting cells also longer peptides are very suitable for the present invention. Thus the whole mutant amino acid sequence may be used as a  
5 frameshift mutant peptide according to the present invention, if it comprises 8 amino acids or more. The invention further relates to a method for vaccination of a person disposed for Alzheimer's disease, consisting of administering at least one peptide of the invention one or  
10 more times in an amount sufficient for induction of T-cell immunity to the mutant  $\beta$ APP and/or Ubi-B proteins.

The invention also relates to a method for treatment of a patient with Alzheimer's disease, consisting of  
15 administering at least one peptide of the invention one or more times in an amount sufficient for induction of T-cell immunity to the mutant  $\beta$ APP and/or Ubi-B proteins.

The invention also relates to a method for treatment of a  
20 patient with Down syndrome, consisting of administering at least one peptide of the invention one or more times in an amount sufficient for induction of T-cell immunity to the mutant  $\beta$ APP and/or Ubi-B proteins.

25

#### Detailed Description of the invention.

In the present description and claims, the amino acids are represented by their one letter abbreviation as known in  
30 the art.

The peptides of the present invention are exemplified by the  $\beta$ APP and Ubi-B frameshift mutations associated with Alzheimer's disease and Down syndrome:

35

In Alzheimer's and Down syndrome patients, intracellular and extracellular deposits of proteins in tangles, neurophil threads and neuritic plaques are correlated with neuronal dysfunction leading to dementia (R.D.Terry et al  
 5 in *Alzheimer Disease*, R.D.Terry, R.Katzman, K.L.Bick, Eds. (Raven, New York, 1994) pp. 179-196). These protein deposits have been shown to contain forms of  $\beta$  amyloid precursor protein ( $\beta$ APP) and ubiquitin-B (Ubi-B) that are aberrant in the carboxyl terminus, and it has further been  
 10 shown that these aberrant protein sequences are results of frameshift mutations which probably occur at the transcriptional level or by posttranscriptional editing of RNA (F.W. van Leeuwen et al, *Science*, vol 279, pp. 242-247).

15 In the case of  $\beta$ APP two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxyadenosine (GA) unit from the (ACC)GAGAGAGA(ATG) sequence in exon 9, and one by deletion of a GA unit from the (CAT)GAGAGA(ATG) sequence in exon 10.  
 20 The mutant  $\beta$ APP peptides resulting from these frameshift mutations are shown in table 1. The peptides with seq id nos 1 and 4 are the mutant part of the  $\beta$ APP protein sequence and the peptides with seq id nos 2, 3 and 5 represent mutant peptides extended into the normal  $\beta$ APP  
 25 sequence at the amino terminus.

normal  $\beta$ APP; RLEAKHRERMSQVMREWEEAERQAKNLPK  
 seq id no 1; NVPGHERMGRGRTSSKELA  
 seq id no 2; RLEAKHRENVPGHERMGRGRTSSKELA  
 30 seq id no 3; RLEAKHRENVPGHERMG  
 seq id no 4; MGRGRTSSKELA  
 seq id no 5; ERMSQVMRMGRGRTS

Table 1.

Also in the case of Ubi-B two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxythymidine (GT) unit from the (TCT)GAGAGGT(GGT) sequence in exon, and one by deletion of a di-nucleoside deoxycytosine-deoxythymidine (CT) unit from the (TCA)CTCT(GGA) sequence in exon 3. The mutant Ubi-B peptides resulting from these frameshift mutations are shown in table 2. The peptides with seq id nos 6 and 9 are the mutant part of the Ubi-B protein sequence and the peptides with seq id nos 7, 8 and 10 represent mutant peptides extended into the normal Ubi-B sequence at the amino terminus.

15

normal Ubi-B;	HLVLRLRGGMQIFVKTLTGKTITLEVEPSD
seq id no 6;	YADLREDPDRQDHHPGSGAQ
seq id no 7;	HLVLRLRGYADLREDPDRQDHHPGSGAQ
seq id no 8;	HLVLRLRGYADLREDPD
seq id no 9;	GCGAQ
seq id no 10;	TLTGKTITGCGAQ

20

Table 2.

25

The mutant  $\beta$ APP and Ubi-B proteins are only encoded for by cells in which corresponding frameshift mutations have occurred and are therefore targets for specific immunotherapy of Alzheimer's disease and Down syndrome.

30

According to the present invention, peptides corresponding to mutant  $\beta$ APP and mutant Ubi-B proteins can be used to elicit T cellular immunity and specific killing of cells producing mutant  $\beta$ APP and mutant Ubi-B proteins, which in Alzheimer's disease and Down syndrome patients are correlated with neuronal dysfunction leading to dementia.

35

Other peptides of the invention can be fragments of the peptides listed in the Tables 1 and 2 above. Such fragments are most preferred from 9-16 amino acids long and include  
5 at least one amino acid from the mutant part of the protein.

As used in this description and claims the term fragment is intended to specify a shorter part of a longer peptide or  
10 of a protein.

### 15 Synthesis

The peptides are synthesised by using continuous flow solid phase peptide synthesis. N-a-Fmoc-amino acids with appropriate side chain protection are used. The Fmoc-amino  
20 acids are activated for coupling as pentafluorophenyl esters or by using either TBTU or di-isopropyl carbodi-imide activation prior to coupling. 20% piperidine in DMF is used for selective removal of Fmoc after each coupling. Cleavage from the resin and final removal of side  
25 chain protection is performed by 95% TFA containing appropriate scavengers. The peptides are purified and analysed by reversed phase (C18) HPLC. The identity of the peptides is confirmed by using electro-spray mass spectroscopy (Finnigan mat SSQ710).

30

Several other well known methods can be applied by a person skilled in the art to synthesise the peptides.

The peptides of the invention may be used in a method for  
35 the treatment of Alzheimer's disease and Down syndrome patients with cells producing frameshift mutant  $\beta$ APP and Ubi-B proteins, which treatment comprises administering at



least one peptide of the present invention *in vivo* or *ex vivo* to a patient in need of such treatment.

5 In another embodiment the peptides of the invention may be used to vaccinate a human being disposed for Alzheimer's disease, by administering at least one peptide of the present invention to said human being.

10 It is further considered to be an advantage to administer to a human a mixture of the peptides of this invention, whereby each of the peptides of the invention can bind to different types of HLA class I and/or class II molecules of the individual.

15 It is considered that the peptides may be administered together, either simultaneously or separately, with compounds such as cytokines and/or growth factors, i.e. interleukin-2 (IL-2), interleukin-12 (IL-12), granulocyte macrophage colony stimulating factor (GM-CSF), or the like  
20 in order to strengthen the immune response as known in the art.

The peptides according to the present invention can be used in a vaccine or a therapeutical composition either alone or  
25 in combination with other materials, such as for instance standard adjuvants or in the form of a lipopeptide conjugate which as known in the art can induce high-affinity cytotoxic T lymphocytes, (K. Deres, Nature, Vol.342, (nov.1989)).

30

The peptides according to the present invention may be useful to include in either a peptide or recombinant fragment based vaccine.

The peptides according to the present invention can be included in pharmaceutical compositions or in vaccines together with usual additives, diluents, stabilisers or the like as known in the art.

5

According to this invention, a pharmaceutical composition or vaccine may include the peptides alone or in combination with at least one pharmaceutically acceptable carrier or diluent.

10

Further a vaccine or therapeutical composition can comprise a selection of peptides which are fragments of the mutant  $\beta$ APP and Ubi-B proteins associated with Alzheimer's disease and Down syndrome.

15

The vaccine according to this invention may further be administered to the population in general for example as a mixture of peptides giving rise to T cell immunity against cells in which Alzheimer's disease and Down syndrome connected  $\beta$ APP and Ubi-B frameshift mutations may occur.

20

The peptides according to this invention may be administered as single peptides or as a mixture of peptides. Alternatively the peptides may be covalently linked with each other to form larger polypeptides or even cyclic polypeptides.

25

30

A therapy for Alzheimer's disease and Down syndrome according to the present invention may be administered both in vivo or ex vivo having as the main goal to elicit specific T cell immunity against the mutant  $\beta$ APP and Ubi-B gene products associated with Alzheimer's disease and Down syndrome.

Further, the frameshift mutant peptides of this invention may be administered to a patient by various routes including but not limited to subcutaneous, intramuscular, intradermal, intraperitoneal, intravenous or the like. In one embodiment the peptides of this invention are administered intradermally. The peptides may be administered at single or multiple injection sites to a patient in a therapeutically or prophylactically effective amount.

The peptides of this invention may be administered only once or alternatively several times, for instance once a week over a period of 1-2 months with a repeated sequence later, all according to the need of the patient being treated.

The peptides of this invention can be administered in an amount in the range of 1 microgram (1  $\mu$ g) to 1 gram (1g) to an average human patient or individual to be vaccinated. It is preferred to use a smaller dose in the range of 1 microgram (1  $\mu$ g) to 1 milligram (1 mg) for each administration.

The invention further encompasses DNA sequences which encodes a frameshift mutation peptide.

The peptides according to the invention may be administered to an individual in the form of DNA vaccines. The DNA encoding these peptides may be in the form of cloned plasmid DNA or synthetic oligonucleotide. The DNA may be delivered together with cytokines, such as IL-2, and/or other co-stimulatory molecules. The cytokines and/or co-stimulatory molecules may themselves be delivered in the form of plasmid or oligonucleotide DNA. The response to a DNA vaccine has been shown to be increased by the presence

of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula :

5'-purine-purine-CG-pyrimidine-pyrimidine-3'. Our DNA vaccines may therefore incorporate these or other ISS, in the DNA encoding the peptides, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, *Immunology Today*, 19(2), 89-97).

10

In one embodiment, the DNA sequence encoding the mutant  $\beta$ APP and mutant Ubi-B peptides comprises:

Normal  $\beta$ APP gene sequence (exons 9 and 10).

15

repeat 1

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG

repeat 2

AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA

20 Mutant  $\beta$ APP gene sequence, GA deleted from repeat 1.

GAG AGG CTT GAG GCC AAG CAC CGA GAG AAT GTC CCA GGT CAT GAG

AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Mutant  $\beta$ APP gene sequence, GA deleted from repeat 2.

25 GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG

AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Normal Ubi-B gene (exon) sequence.

deletion motif

30 CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG

ACC CTG ACC GGC AAG ACC ATC ACC CTG GAA GTG GAG CCC AGT GAC

Mutant Ubi-B gene sequence, GT deleted from the deletion motif.

CAC CTG GTC CTG CGT CTG AGA GGG TAT GCA GAT CTT CGT GAA GAC  
CCT GAC CGG CAA GAC CAT CAC CCT GGA AGT GGA GCC CAG TGA

5

Normal Ubi-B gene (exon 2) sequence.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG  
CT repeat

ACC CTG ACC GGC AAG ACC ATC ACT CTG GAG GTG GAG CCC AGT GAC

10

Mutant Ubi-B gene sequence, CT deleted from the CT repeat.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG  
ACC CTG ACC GGC AAG ACC ATC ACT GGA GGT GGA GCC CAG TGA

15

The invention further encompasses vectors and plasmids comprising a DNA sequence encoding at least one frameshift mutant  $\beta$ APP and/or Ubi-B peptide. The vectors include, but are not limited to *E. Coli* plasmid, a *Listeria* vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40, bovine papilloma virus and the like comprising the DNA sequence encoding a mutant  $\beta$ APP and/or Ubi-B peptide.

It is considered that a treatment for Alzheimer's disease and Down syndrome, or prophylaxis for Alzheimer's disease, may be achieved also through the administration of an effective amount of a recombinant virus vector or plasmid comprising at least one insertion site containing a DNA sequence encoding a frameshift mutant peptide to a patient,

whereby the patient's antigen presenting cells are turned into host cells for the vector/plasmid and presentation of HLA/frameshift mutant peptide complex is achieved.

- 5 A person skilled in the art will find other possible use combinations with the peptides of this invention, and these are meant to be encompassed by the present claims.

10 The peptides according to this invention may be produced by conventional processes as known in the art, such as chemical peptide synthesis, recombinant DNA technology or protease cleavage of a protein or peptide encoded by frameshift mutated  $\beta$ APP gene and Ubi-B gene. One method for chemical synthesis is elucidated in the description  
15 below.

Through the present invention the following advantages are achieved:

- 20 It offers a possibility to treat patients suffering from Alzheimer's disease and Down syndrome connected with frameshift mutant  $\beta$ APP and Ubi-B gene products, who known at present do not have any good treatment alternatives.
- 25 Furthermore it offers a possibility to vaccinate humans prophylactically against the onset of Alzheimer's disease.

Claims

1. A peptide characterised in that it

5 a) is at least 8 amino acids long and is a fragment of a mutant  $\beta$ APP and/or Ubi-B protein arising from a frameshift mutation associated with Alzheimer's disease and/or Down syndrome;

10 and

b) consists of at least one amino acid of the mutant part of the mutant  $\beta$ APP and/or Ubi-B protein;

15 and

c) comprises 0-10 amino acids corresponding to the carboxyl terminus of the normal part of the protein sequence preceding the amino terminus of the mutant sequence and may  
20 further extend to the carboxyl terminus of the mutant part of the protein as determined by a new stop codon generated by the relevant frameshift mutation;

and

25

d) induces, either in its full length or after processing by antigen presenting cells, T cell responses.

2. A peptide according to claim 1 characterised in that it  
30 contain 8-25 amino acids.

3. A peptide according to claim 1 characterised in that it contain 9-20 amino acids.

4. A peptide according to claim 1 characterised in that it contain 9-16 amino acids.
5. A peptide according to claim 1 characterised in that it  
5 contain 8-12 amino acids.
6. A peptide according to claim 1 characterised in that it contain 20-25 amino acids.
- 10 7. A peptide according to claim 1 characterised in that it contains 9 amino acids.
8. A peptide according to claim 1 characterised in that it contains 12 amino acids.
- 15 9. A peptide according to claim 1 characterised in that it contains 13 amino acids.
10. A peptide according to claim 1 characterised in that it  
20 is selected from a group of peptides having the following sequence identity numbers:  
seq id no. 1 - seq id no. 10 or a fragment of any of these.
11. A pharmaceutical composition comprising a peptide  
25 according to any of the above claims and a pharmaceutically acceptable carrier or diluent.
12. A vaccine for Alzheimer's disease comprising a peptide  
30 according to any of the claims 1-10 and a pharmaceutically acceptable carrier or diluent.
13. Use of a peptide according to any of the claims 1-10  
for the preparation of a pharmaceutical composition for  
treatment or prophylaxis of Alzheimer's disease or  
35 treatment of Down syndrome.



14. Method for vaccination of a person disposed for or afflicted with Alzheimer's disease, consisting of administering at least one peptide according to the claims 1-10, one or more times, in an amount sufficient for  
5 induction of specific T-cell immunity to mutant  $\beta$ APP and/or mutant Ubi-B peptides associated with Alzheimer's disease and/or Down syndrome.
15. Method according to claim 14 wherein the amount of the  
10 peptides is in the range of 1 microgram (1  $\mu$ g) to 1 gram (1g) and preferentially in the range of 1 microgram (1  $\mu$ g) to 1 milligram (1 mg) for each administration.
16. Method for treatment of a patient afflicted with  
15 Alzheimer's disease or Down syndrome, by stimulating in vivo or ex vivo with peptides according to the claims 1-10.
17. Method according to claim 16 wherein the amount of the  
20 peptides used is in the range of 1 microgram (1  $\mu$ g) to 1 gram (1g) and preferentially in the range of 1 microgram (1  $\mu$ g) to 1 milligram (1 mg) for each administration.
18. An isolated DNA sequence comprising a DNA sequence or  
25 variants thereof encoding a frameshift mutant peptide according to claim 1.
19. An isolated DNA sequence according to claim 18 encoding peptides comprising seq. id. no: 1-10 or variants thereof.
- 30 20. Use of a DNA sequence according to any of the claims 18-19 for the preparation of a pharmaceutical composition for treatment or prophylaxis of Alzheimer's disease or treatment of Down syndrome.

21. Method for treatment of a person disposed for or afflicted with Alzheimer's disease or afflicted with Down syndrome, by stimulating *in vivo* or *ex vivo* with DNA sequences according to the claims 18-19.

5

22. A plasmid or virus vector comprising DNA sequences of claim 18 encoding a frameshift mutant  $\beta$ APP peptide and/or Ubi-B peptide associated with Alzheimer's disease or Down syndrome.

10

23. A vector according to claim 22 wherein the vector is *E. Coli* plasmid, a *Listeria* vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40 or bovine papilloma virus.

15

24. Use of a plasmid or virus vector according to claim 22 for the preparation of a pharmaceutical composition for treatment or prophylaxis of Alzheimer's disease or treatment of Down syndrome.

20

25. Method for treatment of a person disposed for or afflicted with Alzheimer's disease or afflicted with Down syndrome, by stimulating *in vivo* or *ex vivo* with plasmids or virus vectors according to claim 22.

25

SEQUENCE LISTINGCOMMON FOR ALL SEQUENCES.

SEQUENCE TYPE: Peptide

SEQUENCE UNIT: Amino Acid

TOPOLOGY: Linear

## SEQUENCE ID NO: 1

SEQUENCE LENGTH: 19 amino acids

N V P G H E R M G R G R T S S K E L A

1            5            10            15

## SEQUENCE ID NO: 2

SEQUENCE LENGTH: 27 amino acids

R L E A K H R E N V P G H E R M G R G R T S S K E L A

1            5            10            15            20            25

## SEQUENCE ID NO: 3

SEQUENCE LENGTH: 17 amino acids

R L E A K H R E N V P G H E R M G

1            5            10            15

## SEQUENCE ID NO: 4

SEQUENCE LENGTH: 12 amino acids

M G R G R T S S K E L A

1            5            10

## SEQUENCE ID NO: 5

SEQUENCE LENGTH: 15 amino acids

E R M S Q V M R M G R G R T S

1            5            10            15

SEQUENCE ID NO: 6

SEQUENCE LENGTH: 20 amino acids

Y A D L R E D P D R Q D H H P G S G A Q  
1            5            10            15            20

SEQUENCE ID NO: 7

SEQUENCE LENGTH: 28 amino acids

H L V L R L R G Y A D L R E D P D R Q D H H P G S G A Q  
1            5            10            15            20            25

SEQUENCE ID NO: 8

SEQUENCE LENGTH: 17 amino acids

H L V L R L R G Y A D L R E D P D  
1            5            10            15

SEQUENCE ID NO: 9

SEQUENCE LENGTH: 5 amino acids

G G G A Q  
1            5

SEQUENCE ID NO: 10

SEQUENCE LENGTH: 13 amino acids

T L T G K T I T G G G A Q  
1            5            10

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NO99/00141

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **14-17, 21 and 25**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**See extra sheet**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NO99/00141

Claims 14-17,21 and 25 relates to methods of treatment of the human or animal body by therapy practised on the human or animal body/ Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

28/09/99

International application No.

PCT/NO 99/00141

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9712992 A2	10/04/97	AU 7142796 A GB 9520080 D	28/04/97 00/00/00
WO 9532731 A2	07/12/95	AU 2623795 A EP 0762891 A GB 9410922 D JP 10504702 T	21/12/95 19/03/97 00/00/00 12/05/98
WO 9845322 A2	15/10/98	AU 7071598 A	30/10/98